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Final Report for Grant No. NCC 2-534

Title: Towards Self-Replicating Chemical Systems Based on Cytidylic and Guanylic Acids

Cooperative Agreement NCC 2-534

Technical Monitor : Sherwood Chang, Chief
Exobiology Branch
MS : 239-4
NASA/Ames Research Center
Moffett Field, CA 94035-1000

Principal Investigator: Anastassia Kanavarioti
Associate Research Chemist
Department of Chemistry and Biochemistry
University of California, Santa Cruz
Santa Cruz, CA 95064
phone: 408-459-2978
FAX: 408-459-2935
e-mail : tessi@hydrogen.ucsc.edu

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Final Report

This project was aimed towards a better understanding of template-directed reactions and, based on this, towards the development of more efficient non-enzymatic, polynucleotide synthesizing systems. These systems could serve as models for the prebiotic synthesis of an RNA world. The major objectives of this project were: (a) To elucidate the mechanistic aspects of template-directed (TD) chemistry, (b) to identify the conditions, environmental and other, that favor "organized chemistry" and stereoselective polymerization of nucleotides and (c) to search and, hopefully, find catalysts that will improve the efficiency of these reactions. Enhanced efficiency is expected to facilitate the road towards a self-replicating chemical system based on all four nucleic acid bases.

During the 3-year granting period from January 1994 to 1997, we have made substantial progress in the first two objectives and do the ground work for the third objective. The research resulted in six papers (#1-6 in the publications list below), one submitted manuscript (#7) and several preliminary results which led to the formulation of a successful proposal. The new granting period started in January 15th of 1997.

The first objective, i.e. the elucidation of the mechanism of TD chemistry, was investigated using the poly(C)/2-MeImpG system. Although this system is very limited, our studies were the first to establish a mechanism for TD chemistry. We learned that, a necessary but most likely not sufficient, requirement for efficient polymerization is the accumulation of long stacks of monomeric units at complementary sites on the template (# 4 in the publications list). Another conclusion reached is that the ratio between elongation rate and dimerization rate determines polymerization efficiency. In other words, larger ratio yields longer oligomers (# 5 in the publications list). These conclusions were supported both by the experiments done during this granting period as well as by computer simulations performed during an earlier granting period. The above notions led to the realization that, besides guanosine, most other bases and especially uridine, may not fullfill the necessary requirement under typical conditions. Therefore, we initiated experiments in highly concentrated aqueous solutions of activated mononucleotides. This highly concentrated solutions simulate the evaporating lagoon scenario that was recently exploited by Robertson and Miller for the efficient prebiotic synthesis of the pyrimidines (see reference Robertson, M. P. & Miller, S. L. An efficient prebiotic synthesis of cytosine and uracil. *Nature*

375, 772-774 (1995)). This new environment, we believe, fullfills the 2nd objective of this project as outlined earlier.

In a nutshell, we have discovered that in this new "environment ", i.e. in highly concentrated (up to 1.5 M) aqueous solutions of mixtures of mononucleotides, the individual components exhibit enhanced solubility. This enhanced solubility strongly suggests intermolecular interactions, most likely stacking. Evidence for interaction is also obtained by analysis of the products and stacking is proposed to occur even with uridine that was not expected to stack. Interestingly, mixtures of reactive mononucleotides exhibit higher percent yields of RNA-type dimers than the equivalent self-condensations, i.e. reactions of a single nucleotide. The latter points to a "missing link" between prebiotic chemistry and the RNA world on which I elaborate in paper # 7 of my publication list. In addition, in the concentrated solutions, the oligomerization reaction is much more favored than the hydrolysis and therefore substantial amounts of oligomers are formed (see #6 of publications list).

Finally, although we have not yet discovered a catalyst for TD chemistry, the work described in papers # 2 and 3 provide the groundwork for further investigations in a promising line of potential prebiotic catalysts, the polyamines. This work is relevant to the third objective of this project.

List of publications that resulted from work during the 3-year granting period 1994-1997.

1. A. Kanavarioti, "Template-directed Chemistry and the Origins of the RNA World," *Origins of Life* **24,** 479-495 (1994).
2. A. Kanavarioti, M. W. Stronach, R. J. Ketner and T. B. Hurley "Large Steric Effect in the Substitution Reactions of Amines with Phosphoimidazolide Activated Nucleosides," *J. Org. Chem.*, **60,** 632-637 (1995).
3. A. Kanavarioti, E. E. Baird and P. J. Smith, "Use of Phosphoimidazolide Activated Guanosine to Investigate the Nucleophilicity of Spermine and Spermidine," *J. Org. Chem.*, **60,** 4873-4883 (1995).
4. A. Kanavarioti, T. B. Hurley, and E. E. Baird, "Affinity of Guanosine Derivatives for Polycytidylate Revisited," *J. Mol. Evol.*, **41,** 161-168 (1995).
5. A. Kanavarioti and E. E. Baird, "Faster Rates with Less Catalyst in Poly(C)-directed Oligoguanylate Synthesis," *J. Mol. Evol.*, **41,** 169-173 (1995).
6. A. Kanavarioti, "Dimerization in Highly Concentrated Solutions of Phosphoimidazolide Activated Mononucleotides," *Origins Life Evol. Biosph.* (1997), in press.
7. A. Kanavarioti, "Enhanced 3',5' Regioselectivity in the Aqueous Dimerizations with Mixtures of Phosphoimidazolide Activated Nucleotides," *J. Am. Chem. Soc.*, submitted.

Please note that papers #6 and 7 resulted from work conducted during the period of September 1995 to January 14, 1997; earlier reports cover the period until August 1995.

Because of the absence of reprints for papers # 6 and 7, we have included the abstracts of these papers at the end of this report.

Progress Report covering the period of September 1995 to January 1997

Participation in meetings:

As part of the activities during the above period of performance the PI attended the following meetings and gave presentations: (i) Invited chairperson at the Gordon Research conference on the Origin of Life in Ventura, CA on January 7-12, 1996. (ii) Invited speaker at the Organic/Inorganic Chemistry Seminar Series at the Department of Chemistry and Biochemistry of the University of California at Santa Cruz on April 22, 1996. (iii) Invited discussion moderator at the Origin of Life Symposium of the NSCORT at NASA/Ames Research Center, on April 18-19, 1996. (iv) Invited chairperson at the 8th ISSOL meeting on the Origin of Life in Orleans, France, on July 7-12, 1996. (v) Two consecutive seminars in the weekly Physical Organic Proseminar at UCSC on Jan 8 and 15, 1997.

Highlights of Accomplishments :

* A new microenvironment has been discovered, see 1st paragraph of the 2nd page of this report, in which mixtures of activated mononucleotides (guanosine, cytidine and uridine) yield mixed oligomers up to 6-mer. This environment does not favor polypurine over polypyrimidine condensation and, more importantly, leads to relatively high percent of RNA dimers.
* We have successfully resolved and identified the products of condensation reactions occuring in the presence of three activated nucleotides (guanosine, cytidine and uridine) by high performance liquid chromatography (HPLC). This task has never been done before, mainly because of the large number of products anticipated; more than 40 to account for the monomeric and dimeric products only.

Abstract for # 6. Phosphoimidazolide activated ribomononucleotides (*pN) are useful substrates for the non-enzymatic synthesis of polynucleotides. However, dilute neutral aqueous solutions of *pN typically yield small amounts of dimers and traces of polymers; most of *pN hydrolyzes to yield nucleoside 5'-monophosphate. Here we report the self-condensation of nucleoside 5'-phosphate 2-methylimidazolide (2-MeImpN with N = cytidine, uridine or guanosine) in the presence of Mg^{2+} in concentrated solutions, such as might have been found in an evaporating lagoon on prebiotic Earth. The product distribution indicates that oligomerization is favored at the expense of hydrolysis. At 1.0 M, 2-MeImpU and 2-MeImpC produce about 65 % of oligomers including 4 % of the 3',5'-linked dimer. Examination of the product distribution of the

three isomeric dimers in a self-condensation allows identification of reaction pathways that lead to dimer formation. Condensations in a concentrated mixture of all three nucleotides (U,C,G mixtures) is made possible by the enhanced solubility of 2-MeImpG in such mixtures. Although percent yield of internucleotide linked dimers is enhanced as a function of initial monomer concentration, pyrophosphate dimer yields remain practically unchanged at about 20 % for 2-MeImpU, 16 % for 2-MeImpC and 25 % of the total pyrophosphate in the U,C,G mixtures. The efficiency by which oligomers are produced in these concentrated solutions makes the evaporating lagoon scenario a potentially interesting medium for the prebiotic synthesis of dimers and short RNAs.

Abstract for # 7: Phosphoimidazolide activated ribomononucleotides (*pN) are useful substrates for the non-enzymatic synthesis of oligonucleotides. In the presence of metal ions dilute neutral aqueous solutions of *pN (0.01 M) typically yield small amounts of dimers and traces of oligomers; most of *pN hydrolyzes to yield nucleoside 5'-monophosphate. We now report an investigation of the reactions of *pN in concentrated aqueous solutions (≈ 1.0 M) in which we found substantial yields of condensation products, including the 3',5'-linked, RNA dimers. The self-condensation of nucleoside 5' phosphate 2-methylimidazolide (2-MeImpN with N = cytidine (C), uridine (U) or guanosine (G)) and the reactions in the three binary mixtures as well as in the ternary mixture were studied in the presence of Mg^{2+} or Mn^{2+}. The percent yield of all major products was determined. It was found that under identical conditions, including the same total monomer concentration, the reactions in the ternary, i.e., U,C,G-mixture, produce the highest yield (≈ 9 %) in RNA dimers, whereas the self-condensations do not yield more than 4 % and the binary (U,C-, C,G- and U,G-mixture) produce dimer yields that fall between 9 % and 4 %. The implications of these results for the RNA world hypothesis are discussed.